IMMUNOLOGICAL AND EXPERIMENTAL STUDIES ON PNEUMOCOCCUS AND STAPHYLOCOCCUS ENDOCARDITIS ("*chronic septic endocarditis*").*†

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INTRODUCTION.

THE factors which determine the localization of bacteria upon the endocardium in endocarditis and their maintenance there are still obscure. In the present article are recorded the results of observations upon the immunological reactions of bacteria isolated from cases of endocarditis, and upon experimental endocarditis. These results indicate that the production of endocarditis by staphylococci and pneumococci, as well as its character and course, depends to a greater degree than heretofore known upon certain acquired and peculiar properties of the bacteria in question.

STAPHYLOCOCCUS WITH SPECIAL CHARACTERISTICS FROM A CASE OF ENDOCARDITIS.

Case 334.—Salesman, 36. No history of previous infection; no rheumatism. Five months previous to beginning of present illness there was ulceration of first right lower molar tooth which was treated by a dentist. The tooth was crowned but the crown had to be removed a month later to let out pus and was soon replaced without further trouble until a week before death.

January 23, 1908, the patient first consulted Dr. Slaymaker for an acute attack of rhinitis and pharyngitis with severe muscular pains, backache, and prostration. A probable diagnosis of influenza was made, but the patient grew worse and entered the hospital four days later. At that time the spleen was palpable but there was no

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agglutination of typhoid bacilli by the serum. No heart murmur was detected. The leucocyte count was 7,950, four days later 12,380; hb. 76 per cent. No malarial parasites. The urine was normal.

February 9, 1908: Severe pain over kidneys and right side posteriorly requiring morphine for relief. Urine previously normal now contains albumin and a large amount of pus, many leucocytic, granular, and blood casts. There are numerous small petechial hemorrhages over the skin associated with a distinct systolic murmur transmitted to the left.

From this time the patient rapidly failed, the picture being that of a rapidly increasing infection. Three days before death he developed a left hemiplegia which improved the day before death. The petechial hemorrhages in the skin became so numerous that a large part of the body was covered with them. Conjunctival hemorrhages also were present. Two blood cultures[1] during life and cultures after death gave a peculiar staphylococcus which is described later. The pulse, temperature, opsonic index, and leucocyte counts illustrate well the rapidly increasing infection.

Anatomic diagnosis.—Alveolar abscess; acute mitral ulcerative endocarditis; embolism of the mesenteric artery with mycotic aneurysm; thrombosis of the splenic vein; multiple infarcts of the spleen and kidneys; cloudy swelling and fatty degeneration of the liver, myocardium, and kidneys; icterus; anemia and emaciation; multiple hemorrhages of the skin, conjunctivae, serous and mucous membranes, endocardium, myocardium, adrenals, and kidneys; hyperplasia of the mesenteric lymph glands and spleen; lobular pneumonia; emphysema of the lungs.

The body is 178 cm. long; poorly nourished. The skin is pale, icteric, still warm. The sclera show numerous punctate and larger hemorrhages. The right first molar is loose and from the alveolus exudes upon pressure a grayish-white pus. The lips are studded with numerous punctate hemorrhages. The genitalia are normal. No edema of the extremities. In the skin everywhere are innumerable pin-head to pea-sized hemorrhages, usually discrete, but at places forming large confluent areas as over the inner surface of the right arm where the area measures 10 cm. across. In the peritoneal cavity a small amount of blood-tinged fluid. The abdominal organs are in normal position, no adhesions. There are numerous petechial hemorrhages in the peritoneum. The mesenteric lymph nodes are enlarged.

The organs in the neck not examined. Mucous membrane of the trachea and the bronchi red and studded with numerous small hemorrhages. Both lungs are free and voluminous, the rounded margins highly emphysematous. The pleurae are smooth and shining. In the right lower lobe, near the interlobular fissure, is an area of consolidation the size of a hen's egg, red and bloody on the cut surface. The upper and middle lobes are edematous. The left lung crepitates throughout except in an area 1 cm. × 8 cm. at the lower border of the lower lobe which is dark gray and moist.

[1] *Blood cultures in endocarditis.*—In this series the blood cultures were made in the usual manner by puncturing one of the large veins at the bend of the elbow and inoculating media with blood. Practically all observers have obtained a higher percentage of positive results in pneumonia, typhoid fever, and septic processes generally by the use of fluid than by the use of solid media. In endocarditis, on the other hand, at least of the type under consideration, agar media have given positive results repeatedly when the cultures in broth and milk remained sterile. For this reason and also because the latter method is a means of learning the number of bacteria in the blood, the importance of inoculating both liquid and solid media in all cases where endocarditis is suspected must be emphasized. Repeated cultures in a number of cases showed that while the bacteremia is constant the number of bacteria is never great. The number of colonies varied between 4 and 2,000 per c.c. of blood.

There is no edema in this lung. The tracheo-bronchial lymph nodes are dark red, soft, and enlarged.

Both visceral and parietal layers of the pericardium are studded with numerous small hemorrhages. There is an irregular cauliflower-like, whitish-gray growth on the mitral valve which plugs the greater portion of the orifice. There are no necrotic areas on the surface of the vegetation. The wall of the left ventricle measures 1 cm. in thickness. The myocardium is soft and yellowish. The tricuspid and semilunar valves are normal. The endocardium is studded everywhere with minute hemorrhages which are very numerous throughout the myocardium as well. The intima of the thoracic and abdominal aorta normal.

The spleen weighs 370 gm. The external surface is smooth, light red except over the many infarcts. The parenchyma soft. The inferior branch of the splenic artery is thrombotic for 1 cm.

Esophagus normal. Stomach moderately dilated; mucous membrane pale red, several ecchymoses in the fundus. Intestinal mucosa shows a number of small as well as large ($\frac{1}{2}$ cm. sq.) hemorrhages.

The liver weighs 1,610 gm., externally grayish brown, more or less granular. Lobular markings distinct. The gall bladder normal. Pancreas normal. The right adrenal weighs 11 gm., the left 7 gm., and both are covered with numerous ecchymotic spots. The kidneys weigh 530 gms. together; the capsules adherent in places. Numerous minute hemorrhages throughout both kidneys. There are also numerous sharply circumscribed, whitish-yellow areas, some of which project from the surface in wedge-like form. The cortex and medulla are sharply defined, the cortex from 6 to 10 mm. thick. Numerous hemorrhages in the pelvis and ureters; only a few in the bladder. Genitalia normal.

The mesenteric branches supplying 80 cm. of the lower portion of the ilium contains an aneurysm as large as a hazel nut filled with thrombus.

Bacteriologic examination.— There were examined the blood from the heart, the vegetations on the mitral valve, the bile, the cerebro-spinal, pericardial, and peritoneal fluids, and pus from the ulcerated tooth. The heart's blood and vegetations gave a Gram positive coccus, single, in small and large clumps. The bile gave a short Gram negative bacillus. The cerebro-spinal and peritoneal fluids were sterile. The pericardial fluid gave a pure culture of the staphylococcus isolated from the blood during life. The pus gave *Staph. albus, Strepto. pyogenes, B. coli,* and a Gram positive bacillus. The staphylococcus from the pus of the tooth acidified and coagulated milk and grew on the surface of agar and in broth and other media exactly as does *Staph. albus.* The characteristics of the strains isolated from the blood on two occasions during life were exactly the same as those of the one isolated after death.

Description of the staphylococcus isolated from the blood.—Upon all media this coccus is of the same size as the pyogenic cocci. It occurs singly, in pairs, and in large clumps. It stains rather slowly with Löffler's methylene blue. It is positive to Gram's stain.

The surface colonies on agar plates are grayish white. They are more elevated, more granular, and more opaque than the colonies of *Staph. abus.* The deep colonies are elongated and opaque. On blood agar a narrow zone of hemolysis surrounds the colonies. Agar streak (aerobic) is grayish white with irregular elevated margin, sticking tightly to the surface so that it is necessary to tear the surface to dislodge the colonies. (This property was lost on cultivation.) Anae-

robically the growth is apparently equally rapid but not so opaque and does not stick to the surface of the medium. In ordinary broth there is a scum on the surface and tenacious, flocculent sediment at the bottom; the intermediate fluid being perfectly clear. Clumps of bacteria adhere closely to the side of test-tube; vigorous shaking fails to dislodge them. After 48 hours the sediment in the bottom may fill half the tube, the media gradually becoming cloudy. After a week the special properties are greatly diminished, and soon they entirely disappear, so that six months later broth cultures produced a diffuse turbidity from the beginning. The anaerobic 48-hour broth culture shows a diffuse turbidity, no scum, only a very small sediment, and no clumps adherent to the sides of the tube. For one month this coccus did not liquefy gelatin when grown aerobically. It liquefied gelatin slowly from the beginning when grown anaerobically. Six months later it liquefied slowly along the line of inoculation. In litmus milk its growth is abundant when grown aerobically but no acid production could be demonstrated until after cultivation for six weeks. After that the milk turned to pink at the end of a week. At present, six months later, the

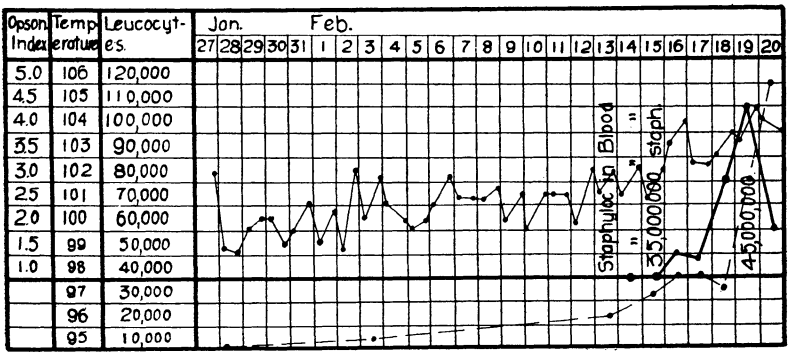

CHART 1.—Opsonic Index, Temperature, and Leucocyte Curves in Case 334. Solid heavy line = pneumococco-opsonic index; solid fine line = temperature; broken fine line = leucocytes.

acid production while still moderate is more marked than formerly. Anaerobic cultures gave moderate acid production from the beginning. The growth on potato and in glucose agar resembles closely that of *Staph. albus*.

Microscopic examination.—In sections of the lung there are patches of broncho-pneumonia, some of considerable extent. In the liver are small regions of fatty changes. The kidney and spleen show infarction and in the myocardium there are scattered minute foci of inflammation.

The observations on Chart 1 and in Tables 1, 2, and 3 concern the foregoing case. The chart gives the pulse, temperature, opsonic, and leucocytic curves. The extremely high leucocyte count of 120,000 was composed of over 90 per cent polymorphonuclears. The opsonin curve should be followed in connection with the injection of killed staphylococci. The injection of 35 million ordinary staphylococci (albus) apparently caused a gradual rise in the index to 4.5 upon the

fourth day. The injection, the day before death, of 45 million killed homologous staphylococci was associated with a prompt drop in the opsonic power. Smears from the blood after death contained numerous staphylococci, many in large clumps, but no evidence of phagocytosis was found although the organism was susceptible to phagocytosis in vitro soon after isolation.

In this case we have a striking example of marked environmental modification of a coccus. The staphylococcus when cultivated was freely susceptible to phagocytosis and possessed practically no virulence to animals in the ordinary sense (Table 4). How then was it possible for the organism to multiply in the patient's blood and endocardium and ultimately produce death? Why should not healing have occurred?

TABLE 1.

RESISTANCE TO PHAGOCYTOSIS AFTER GROWTH IN BLOOD AND SERUM.

MIXTURE	PHAGOCYTOSIS	
	15 Min.	6 Hr.
Washed normal blood + normal serum + Coccus 334 (susp. from agar)	3.74	∞
" " " + 334 " + " " " " "	4.26	∞
Defibr. " " + Coccus 334 from patient's blood *	0	0
" 334 " + " " " " "	0	0
" " " + " " from vegetation	2.8	3
Washed normal blood + " " in patient's serum 48 hrs.	0.12	0.2
" " " + " " in normal serum 48 hrs.	0.25	0.4
" " " + " " from agar	0.02	0.06

* Centrifugalized from fresh serum. ∞ Innumerable.

Table 1 shows that the patient's serum has a normal opsonic index (1.1). The organism used in the determination had been grown on plain agar for only 72 hours after isolation from the blood of the patient, the suspension being from a 24-hour subculture. When the cocci have grown in vivo they resist phagocytosis in defibrinated blood, normal as well as patient's. When grown in sera (normal and patient's) for 48 hours they also acquire a high degree of resistance to phagocytosis. This is an acquired resistance to opsonin and not the result of a change in the serum, because the patient's serum originally gives a normal opsonic index for the organism grown on plain agar. Titration showed no change in the reaction of the serum in which the organism is grown, the organism at this time not producing acid. The serum in which this strain has grown for 48 hours still shows some opsonic power toward organisms grown upon agar. On the other

hand, the organisms obtained from the vegetations are susceptible to phagocytosis in the patient's defibrinated blood. Smears made directly from the vegetations show also evidence of phagocytosis.

TABLE 2.

OPSONIC EFFECT OF SERUM 334 AND NORMAL SERUM UPON STAPHYLOCOCCUS 334.

	Mixtures (All contained equal parts of staphylococcus suspension, washed normal blood [washed three times in 40 times its volume], and serum or NaCl solution)	Phagocytosis
1.	Coccus 334 grown in Serum 334 48 hrs.	0
2.	" " " " heated* " " "	7
3.	" " " " normal serum 48 hrs.	0
4.	" " " " heated " " "	2.4
5.	" " " " Serum 334 and washed + NaCl Sol.	0
6.	" " " " " " " " + normal serum	10
7.	" " " " " " " " + Serum 334	0
8.	" " " " " " " heated & washed + NaCl Sol.	0
9.	" " " " " " " " " + normal serum	6.8
10.	" " " " " " " " " + Serum 334	3.5

*Heated means 65° C. for one hour. The organisms designated as "washed" were washed twice in NaCl solution.

Table 2 summarizes the results obtained by a closer study of this interesting phenomenon. Lines 1 to 4 show that the acquired resistance of the coccus to phagocytosis is closely related to certain heat-sensitive properties of the serum as it resists phagocytosis completely when grown in the unheated serum, but becomes quite freely susceptible when grown in the heated serum, then suspended in washed normal blood and NaCl solution. Line 8 shows that the washed organisms grown in heated serum are insusceptible to phagocytosis when suspended in NaCl solution. The resistance acquired by the coccus when grown in unheated serum seems specific for the opsonins in this serum, because in normal serum it is taken up readily but not in the patient's serum (see lines 5, 6, 7). This is borne out by the results in lines 9 and 10 which show that when grown in heated serum the organism is susceptible to phagocytosis both in normal serum as well as in the patient's. Here we may have the explanation why a coccus of such a mild degree of virulence could live in a body and ultimately produce death, namely, an acquired, specific immunization against the antibodies of the host.

Table 3 shows that normal unheated serum has a bacteriolytic effect on staphylococcus 334 which is destroyed by heating to 60° C. for one hour. On the other hand the coccus grows as well in the unheated as in the heated serum of the patient. The staphylococcus aureus grows equally well in both sera. The patient's serum has a

marked agglutinating effect on strain 334 as have the sera from cases of pneumococcus endocarditis on homologous pneumococci.

TABLE 3.

EFFECT OF NORMAL SERUM AND SERUM 334 UPON STAPHYLOCOCCUS 334 AND *Staph. Aureus*

MIXTURES	AGGLUTINATION	COLONIES ON AGAR PLATES		
		At Once	12 Hrs.	48 Hrs.
Unheated normal serum + Coccus 334	No Agglutination	2500	1350	25
Unheated Serum 334 + " "	Marked "	3000	2800	5000
Heated * normal serum + " "	No "	2300	1800	3100
Heated * Serum 334 + " "	Moderate "	3000	2500	6300
Unheated normal serum + *Staph. aur.*	No "	350	∞	∞
Unheated Serum 334 + " "	Slight "	365	∞	∞

* 60° C. for 1 hr. ∞ Innumerable.

The infection in this case undoubtedly came from the ulcerated tooth. Probably the gradual acquirement of the special properties described, namely, resistance to phagocytosis, adherence to surfaces, and growth in clumps, favored involvement of the endocardium. That the peculiar properties were acquired before the endocardium became involved and that the coccus immunized itself gradually against the antibodies in the patient as the infection progressed are indicated by the fact that as soon as these special properties were lost on artificial cultivation, it was impossible any longer to produce endocarditis experimentally; furthermore, repeated inoculations, instead of producing an immunity, produced hypersensitiveness and the coccus grown in one animal receiving repeated inoculations resisted phagocytosis and formed clumps. The fact that the organism grew aerobically in characteristic manner, but not when under anaerobic conditions, may be the reason why the left side of the heart was involved and not the right side. Intravenous inoculations of cocci grown anaerobically failed to produce lesions in three rabbits soon after the strain was isolated, an indication that the special characteristics were responsible for the promptness with which the coccus produced endocarditis so long as they were present.

ANIMAL EXPERIMENTS WITH STAPHYLOCOCCUS 334.

Table 4 gives the most important results obtained in 12 rabbits inoculated from 5 to 15 days after the organism was isolated; six developed either a pericarditis or endocarditis or both. One of these six was inoculated directly into the heart, three intravenously: one received an intraperitoneal injection and six days later the second injection intravenously; and the other a primary intravenous and two weeks later an intraperitoneal injection. Three of the six that developed no lesions were inoculated

subcutaneously, one intravenously and two intraperitoneally. The two last received two intraperitoneal injections, recovering promptly after the first but succumbing to an acute fibrinous peritonitis after the second. Of the seven guinea-pigs none died after the first inoculation. Attempts to cause suppuration with this organism by subcutaneous injections failed. The second inoculations in two of the animals were ineffective when made intraperitoneally, the animals recovering although not so promptly as after the first injection. On the other hand, a second intraperitoneal injection proved fatal in all of three guinea-pigs tested. One animal received three injections, recovering promptly after the first which was given subcutaneously, more slowly after the second which was given intraperitoneally, but died from acute peritonitis and an overwhelming bacteremia after the second intraperitoneal injection. The time between the first and second injections in these animals varied from 4 to 24 days. On the whole the guinea-pigs proved more resistant than rabbits, none developing endocarditis or pericarditis.

Two dogs were injected, one into the heart and the other intravenously three weeks after the organism had lost part of the special characteristics. The one injected into the heart lost weight and was ill for 10 days, then seemed to get better, and when chloroformed a healing tricuspid and mitral endocarditis was found. The cultures made from the blood were sterile. In the other dog the injection seemed to have no effect.

The special affinity of this organism for the endocardium and pericardium is shown in the rabbit by the high percentage of involvement of these structures independently of the place of inoculation. The tendency in all cases of the endocarditis was to heal; none of the animals died spontaneously from this cause. In one instance of a beginning mitral endocarditis microscopic section of the nodule showed infiltration beneath the layers of the normal endothelium on both sides of the cusps.

Two months after the organism was isolated and when the peculiar properties had been lost, attempts to produce endocarditis and other lesions in rabbits failed. The apparent increased sensitiveness of the guinea-pig and rabbit, the result of relatively small, primary, and quite harmless doses of this organism, merits further mention. A study of the peritoneal smears in these animals showed that after the first inoculation prompt migration and phagocytosis of the cocci took place. Later many endothelial cells appeared which were markedly phagocytic for cocci, and leucocytes, especially those containing cocci, were taken up by the endothelial cells. After the second injection prompt migration of leucocytes and phagocytosis again occurred, which in no way differed from those after the first injection, but instead of the appearance of endothelial cells, phagocytic for leucocytes and cocci,

TABLE 4.

SUMMARY OF EXPERIMENTS WITH STAPHYLOCOCCUS 334.

Animal	Days Since Isolation of Staphylococcus	Inoculation	Postmortem	Bacteriologic Examination	Remarks
Rabbit 9	9	3/1/08—6 c.c. broth culture into heart	3/24/08—Massive pericarditis; mural endocarditis	Coccus 334 isolated from pericardium, endocarditic area, and heart's blood	Died from infection
"	9	3/1/08—5 c.c. broth culture intravenously	3/18/08—Tricuspid endocarditis	Coccus 334 isolated from heart and crushed portion of vegetation	Chloroformed
"	11	3/3/08—6 c.c. 24-hr. broth culture intraperitoneally	4/6/08—Pulmonary, aortic, and mural endocarditis; no peritonitis	Gram positive cocci in vegetation cultures. Blood sterile	Recovered promptly. Chloroformed
"	5	3/9/08—5 c.c. broth culture intravenously		Coccus 334 isolated from nodules on endocardium and heart's blood	Chloroformed
"	15	3/20/08—5 c.c. broth culture intravenously	3/25/08—Tricuspid and mural endocarditis	Coccus 334 isolated from heart's blood and nodules on tricuspid valve	Died from infection
"	15	3/20/08—15 c.c. 24-hr. broth culture intravenously	3/21/08—Tricuspid (beginning) endocarditis	Coccus 334 from heart's blood and pericardium	Died from second injection
"	8	2/28/08—3 c.c. 24-hr. broth culture intravenously	4/12/08—Massive pericarditis and pleuro-pericarditis; no peritonitis. Beginning mitral endocarditis	Coccus 334 from heart's blood and peritoneum	Died from second injection
"	10	3/14/08—5 c.c. broth culture intraperitoneally			
"	8	2/28/08—5 c.c. broth culture intraperitoneally	3/16/08—Fibrinous peritonitis; no endocarditis		
"	13	3/4/08—intraperitoneally			
"	8	2/28/08—6 c.c milk culture intraperitoneally 3/3/08—8 c.c. broth culture intraperitoneally	3/5/08—Acute sero-fibrinous peritonitis; no endocarditis or pericarditis		
Guinea-Pig	5	2/25/08—Growth over surface of one agar slant in NaCl subcutaneously	3/26/08—No endocarditis; no peritonitis. Infarcts of spleen and kidneys		
"	12	3/4/08—5 c.c. broth culture intraperitoneally			Died from third injection
"	22	3/14/08—Same as above		Blood culture 2 days after injection gave coccus 334. Cultures after death sterile	Chloroformed. Sick for 10 days then improved
Dog	24	3/28/08—35 c.c. broth culture into heart	4/16/08—Healing tricuspid and mural endocarditis		

the exudate was thin with fewer leucocytes and few endothelial cells; phagocytosis was diminished and invasion of the blood took place without peritonitis in guinea-pigs, with peritonitis in rabbits. The organisms in the blood in one of the guinea-pigs were so numerous that there was no difficulty in finding them in smears. No evidence of phagocytosis was seen, notwithstanding that there were 15,000 leucocytes per c.mm. On concentration of the cocci by centrifugalization, then adding defibrinated blood and incubating for 15 minutes to three hours, phagocytosis failed to take place. On cultivation the coccus was susceptible to phagocytosis and a single injection into other guinea-pigs was without effect. Hence it would seem that the animals receiving more than one injection died from an acquired susceptibility and not from an increase of virulence of the organism. At the time the injections were made, the special characteristics had been lost, and this may explain the absence of endocarditis.

SUMMARY OF THE CASES OF PNEUMOCOCCUS ENDOCARDITIS.

Full protocols are given only of the cases that were studied closely, or in connection with which animal experiments were made. Clinically all the 14 cases in question were subacute in their course and while they must be regarded as cases of malignant endocarditis in that the patients all succumbed to the infection, they may be classed under the group designated by Osler[1] as chronic septic endocarditis. Four presented the picture of incompetent hearts from old lesions; the existence of an active endocarditis did not seem probable because there was no leucocytosis, practically no fever or other evidences such as petechial hemorrhages, infarcts, etc., to suggest the diagnosis of a more acute endocarditis. The blood culture was the means of making the diagnosis in three of the four cases, while the other was discovered at autopsy.

All but three of the 14 occurred between 15 and 29 years of age. The occupation of all was chiefly indoors. Six were male and eight female. In 10 cases the original source of the infection could not be determined; in two it seemed to be an attack of tonsilitis; in another the most probable source was pyorrhea alveolaris, while in still another it was an alveolar abscess. The exact duration of the disease could not be accurately determined but probably ranged from 4 to 12 months

[1] Osler, "Endocarditis," *Modern Medicine*, Philadelphia, 1908, 4, p. 133.

or more in the different cases. The blood cultures were the means of making an early positive diagnosis in almost all the cases. In some of the cases it was difficult to make a correct diagnosis because of the insidious onset, the chronicity, and most of all because the acute process was engrafted upon an old valvular lesion in seven cases; in five of these the old lesion was due to acute articular rheumatism; in three this point could not be definitely settled, while in four no definite previous heart lesion was present. Of the four last mentioned there was a definite source of infection in two while in the other two there was none. Petechial hemorrhages in the skin occurred late in nearly every one of the cases, long after the blood showed cocci. Probably the most constant feature was the development of a high grade of anemia of the secondary type. This was most pronounced in the cases which ran the more chronic course. A persistent leucocytosis was present in only four of the cases; the rest showed little or only temporary increase in the number of leucocytes. Definite chills occurred only rarely and in no case in which chills seemed to mark the onset of the disease. Early the fever was remittent. In some patients the temperature was practically normal for weeks and then showed an afternoon rise for a short period. Later, as embolism and petechial hemorrhage occurred, the fever took a septic or intermittent type associated with sweats. Definite joint symptoms were present in three of the cases, in none of which did suppuration occur. The relation between these cases and acute articular rheumatism is an interesting one. The coccus found resembles quite closely "*Micrococcus rheumaticus*" of Poynton and Payne, with, however, certain important differences. The facts that joint symptoms were few and mild in these cases; that arthritis developed only once in the animals injected, and that only five of the cases had had a previous acute articular rheumatism would seem to be proof that we are dealing with another form of infection in these cases. The valves on the left side alone were involved in 10 cases; the mitral valve alone seven times; mitral and aortic twice; aortic alone once. The right side alone was involved three times; tricuspid alone twice; pulmonary once. Both sides (aortic and tricuspid) were involved in one case. Autopsies were obtained in six of the cases. Anatomically the valvular lesions were all characterized by huge vege tative growths. The petechial hemorrhages were probably not all caused

by the breaking-loose of necrotic portions of the growths, but also by clumps of cocci too large to go through the capillaries. It should be noted here that necrotic areas on the vegetations were present at autopsy in the cases where other bacteria than the one responsible for the endocarditis were found with the latter in the blood post mortem. In two of the cases (311 and 292) the rapid failure near the end was undoubtedly due to the invasion of streptococci of high virulence. In the latter only hemolysing colonies were found and had not the coccus which was found during life also been found in cultures made from the vegetation after death, it might have led one to believe that these cocci had taken on the property of hemolysing blood as Buerger and Rytten-berg[1] claim to have found.

The infarcts did not show evidences of suppuration.

Case 292.—Girl, 21, single, student, family and previous history of no special significance. Always in good health; no history of tonsilitis or rheumatism; has not menstruated for the past four months, the flow having gradually grown less. Lost endurance and became nervous one year ago after a year's hard study. Improved somewhat during summer vacation and returned to work but had to give it up on account of weakness with some shortness of breath upon exertion; at the same time there was an increasing pallor with a sense of oppression in the chest, associated with a persistent cough, the result, it was then believed, of a severe cold. The temperature for one week at this time ranged between 99° and 102° F. Went south for the winter but failed to regain health; gradually grew worse and took to bed five weeks before death. The temperature now was 102° F.; there was a feeling of tightness and oppression over region of heart, a dry persistent cough, shortness of breath, great weakness and swelling of the abdomen. The blood showed 3,624,000 reds, 55 per cent hb., and 6,500 whites. No abnormal white or red cells were found. The urine remained normal until two weeks before death when it contained much albumin, epithelial, leucocytic, granular, and hyaline casts, and a few red cells.

Examination (five weeks before death).—Marked pallor of skin and mucous membranes; no icterus; no petechial hemorrhages. Throat and tonsils normal; lungs and pleurae normal. Heart enlarged 2 cm. beyond normal line, apex beat diffuse, loud systolic murmur over base with maximum intensity over pulmonic area; no thrill; pulse rapid, quick, easily compressible, of normal volume. The abdomen showed a shifting line of dulness; a mass in the left hypochondrium resembling spleen and somewhat tender, extending to crest of ilium below and to umbillicus on the right. Liver reaches two fingers' breadth below costal arch, left lobe tender. Rectum normal. Tuberculo-opsonic index 0.7; pneumococco-opsonic index 0.6; streptococco-opsonic index 0.7.

The presence of marked anemia of the secondary type with no leucocytosis and with enlarged spleen and liver pointed to Banti's disease. A possible hyperplastic tuberculous peritonitis with a greatly thickened omentum was also considered and the discovery of small and large moist rales over the right apex a short time previously

[1] *Jour. Infect. Dis.*, 1907, 4, p. 609.

supported this possible diagnosis. Blood cultures (suggested by Dr. Billings) gave a pneumococcus with certain peculiarities to be described, and a diagnosis of acute endocarditis was now made.

The patient grew worse slowly until four days before death when she had a pulmonary hemorrhage; she now failed rapidly from a seemingly overwhelming infection.

Anatomic diagnosis.—Acute vegetative and ulcerative endocarditis of the pulmonary valves and artery; subcutaneous and renal hemorrhages; multiple old and recent pulmonary infarcts; serofibrinous pleuritis; cloudy swelling of the heart, liver, and spleen; hyperplastic splenitis; anemia; passive hyperemia of the liver and spleen; hydrothorax; hydroperitoneum and hydropericardium; left fibrous pleuritis.

The body is well developed and medium sized. Pallor of the skin and mucous membranes; marked posterior lividity; rigor mortis. Irregularly distributed over the entire body are small, circumscribed, red spots from 1 to 3 mm. in diameter. The abdomen moderately distended, the cavity contains about two liters of slightly turbid fluid; lining smooth, no adhesions. The diaphragm reaches to upper border of sixth rib on left side and to sixth interspace on right side. The right pleural cavity contains a large amount of sero-fibrinous fluid; no adhesions. The left cavity contains a small amount of almost clear fluid and is partly obliterated by loose fibrous adhesions. The pericardium is smooth and contains 100 c.c. of serous fluid. The tongue normal. The uvula is slightly enlarged and edematous. The tonsils are enlarged. The tracheal lining is reddish. Both lungs are heavy; on the pleural surface of the right lung are several areas varying in size up to that of a small apple, somewhat raised, hard to the touch, and dark red in color; on section they extend as wedges toward the center of the lung and form sharp edges. The left lung has only three such areas, all of about the same size, i. e., 1 to 2 cm. in greatest diameter. The one in the left upper lobe shows a cavity produced by softening opening into a bronchus of the second degree. Over the infarcts the pleura is covered with fibrinous exudate. Both lungs contain much frothy, watery, mucoid fluid. The tracheo-bronchial lymph nodes are normal. The heart is about normal in size; the muscle pale with a yellow tinge; aortic, mitral, and tricuspid valves normal.

Pulmonary valves are covered with large grayish-yellow cauliflower-like vegetations, which nearly fill the lumen and at the base of which valves are ulcerated. Some of these excrescences are 2 cm. long. The intima of the pulmonary artery just above the valve also is ulcerated. At the point of bifurcation of the artery is a thrombus 0.75 cm. thick, solidly attached and readily followed to the periphery of the left upper lobe where it ends in the softened infarct just described. Several other small branches in the left lung are thrombosed. In the right lung is also extensive thrombosis and the branch leading to the largest infarct which is in the lower lobe is plugged throughout. On removing the thrombi from some of the smaller branches of the pulmonary artery the intima appears rough, even ulcerated.

The spleen is 27×14×7 cm. in size, capsule smooth, consistency somewhat uneven, dark blood on section. The liver shows a pale, yellowish parenchyma with indistinct markings and numerous pin-head ecchymoses. Gall-bladder normal. Also pancreas and adrenals. The kidneys are normal in size; capsule strips easily, leaving a smooth pale surface. The substance is soft. Genital organs normal.

Bacteriological examination:—The peritoneal, pleural, and pericardial fluids and heart's blood, plated on blood agar, yield a large number of grayish colonies surrounded, at the end of 24 hours, by a wide hemolytic zone, from 3 to 5 mm. in width depending

on the number of colonies present in the plate. Two drops of the material (including the blood) yield a countless number of colonies. No acid in inulin broth and the morphology and staining reactions are those of *Strept. pyogenes* and 2.5 c.c. of a 24-hour broth culture of this organism obtained from the blood killed a rabbit weighing 1,350 grams in 48 hours. Pure culture of streptococcus was obtained also from the spleen, liver, pancreas, heart, and from the recent, large area of infarction. In the softened infarct the staphylococcus aureus was found also. The bile contained the colon bacillus. Emulsions from vegetation on the tricuspid valve and the right ventricle contained both streptococci and pneumococci, the former outnumbering the latter 50 fold upon blood-agar plates. The pneumococcus-like colonies tended to adhere to the surface of blood agar, fermented inulin slowly, and at first the virulence was slight, a rabbit weighing 1,250 grams succumbing at the end of five days with a serofibrinous peritonitis and bacteremia after injection of a huge dose. No endocarditis was found. This organism showed capsules when first isolated. The strains obtained after death as well as one isolated from the blood before death were susceptible to phagocytosis. Plates from broth cultures of a strain obtained during life gave smaller and larger colonies of Gram positive diplococci and subcultures of the large colonies produced only large colonies and those of the smaller colonies yielded only small colonies. Three other strains of pneumococci obtained from protracted pneumococcal endocarditis all showed this peculiarity; strains from four cases of acute pneumococcal endocarditis failed to show it, the colonies being all large, and produced more green than the ones isolated from the chronic cases. Similar results are obtained at times with pneumococci obtained from the blood in pneumonia after long cultivation on the same artificial medium and after the strain begins to lose its vigor.

Case 293.—Clerk, age 21, admitted April 23, 1907, into the service of Dr. Billings at the Presbyterian Hospital. Had an attack of acute articular rheumatism three years ago. At this time he began to have heart trouble and ever since had shortness of breath on exertion, associated with palpitation of the heart and precordial pain. Denied venereal infection. Five months previously the patient had severe headache for a few days; was weak and had no appetite. A month later he began to have a dull pain in the palm of the left hand and later pain appeared in the region of the left hip. The pain in the hip lasted for a few days and then went away. Has had some pain but no swelling in left knee and ankle from time to time. During this time his general health was poor, no appetite, sleeplessness, nervousness, lost 30 pounds in weight. Two weeks previously developed severe pain in the left hip associated with a pulsating swelling above and behind the trochanter.

Physical examination on admission shows nothing of note except as regards the vascular system: Apex beat 5 interspace just inside nipple line, no thrill; dulness extends from 1 cm. to left of mammary line to right sternal border, above to third rib. A very distinct and loud diastolic murmur is heard best over aortic area and along sternum downward but not transmitted along vessels of neck and heard only faintly at apex. Capillary pulse present in fingers. Regular water-hammer pulse.

The spleen descends 2 cm. below costal margin; not tender, rather firm.

Over the anterior part of the left hip and a little behind the great trochanter may be seen and felt a pulsating heaving mass. On palpation a distinct thrill is felt; the pulsation is synchronous with the heart beat. The mass is distinctly tender, not very firm, and over it is heard a distinct bruit synchronous with pulse. Pulse in both popliteal arteries equal in volume and force. X-ray picture shows nothing abnormal.

On admission a blood count gave red corpuscles 4,336,000; leucocytes 6,900; hemoglobin 71 per cent. Differential white count: Small mononuclears, 10.8 per cent; large mononuclears, 4.3 per cent; polymorphonuclear neutrophiles, 85 per cent; no eosinophiles or myelocytes.

April 29, 1907: Mass in left hip is much more extensive and more boggy to the touch. It is now difficult to get any pulsation and bruit is heard only indistinctly.

May 12, 1907: Needle introduced into aneurysm brings pure bright red blood, under strong pressure; and cultures from this gave the same organism as obtained from the blood previously.

June 16, 1907: Small pulsating mass as large as walnut under the left jaw; tender, rather soft; no distinct fluctuation, no redness.

June 24, 1907: A small petechial spot on right side near costal margin; another in the right conjunctiva. The hemoglobin 50 per cent.

July 19, 1907: Suddenly developed right hemiplegia and coma. Cannot move lower part of right face; wrinkles forehead nearly equally; closes and opens eyes. Eyes turned to left but can roll them to right. Cannot protrude tongue; right arm and leg absolutely immovable. Moves left arm and leg. Breathing stertorous. Heart condition not changed. Death.

All of four blood cultures, obtained in the usual manner, and repeated cultures from puncture of the aneurysm gave pure growths of a variably sized Gram positive diplococcus which in the early cultures in broth and milk formed short chains and clumps. Upon continued cultivation all of the strains have lost the tendency to grow in clumps and now grow in typical diplococcus form; the strains isolated first lost this property more rapidly than the ones isolated later in the course of the disease. The organism grew in large clumps in the fibrin clot, adhered moderately to the surface of the blood-agar slants when first isolated, and produced small colonies in blood-agar plates with only a slight greenish zone about them. No hemolysis occurred when normal or homologous blood was used in the plates. Two strains tested fermented inulin slowly, a property which they soon lost upon cultivation. The organisms were susceptible to phagocytosis in normal and homologuos blood soon after isolation. The injection of rabbits showed that this was associated with a very low grade of virulence, both animals, each of which received 5 c.c. of a 24-hour broth culture intraperitoneally, recovering promptly. Here may be noted that the smears from the blood in the aneurysm showed a large number of diplococci and leucocytes but no definite evidence of phagocytosis. The size of the organism and the character of the colonies produced on blood agar resembled closely those sometimes produced by pneumococci which have been isolated from the blood in pneumonia and which have lost their vigor from long cultivation on artificial media.

Case 311.—F. S., girl 18 years old. Had always been in good health except for an attack of acute articular rheumatism six years ago, in which she developed mitral lesions. Admitted to the Presbyterian Hospital, October 28, 1907, under Dr. Sippy, to whom I am indebted for the opportunity of studying the case. Thanks are also due Dr. Pickering for the opportunity of studying the case after the patient left the hospital. Three months before admission the patient had an attack of severe sticking pain in the right chest, made worse by deep breathing and coughing. She had fever but no cough, no hematemesis. She remained in bed for a week, but never regained her former health and had a continued evening temperature of about 101° F. Three weeks before admission the patient noticed that her left ankle was swollen but not

painful. The swelling came on gradually and left in a week. No other joints have been swollen. On admission she complains of chilliness at times, prostration, and malaise. There has never been cough, localized discoloration of the skin, bloody urination, or trouble with the eyes. Bowels are regular; appetite good; sleeps fairly well.

Examination.—Decided pallor; no discolorations of the skin; fairly well nourished; the tonsils are not enlarged and have a normal appearance. Cardiac dulness is not increased to the right but extends just outside of mammillary line to the left. Auscultation reveals a presystolic and systolic murmur with maximum intensity over mitral area and transmitted slightly to the left. Lungs and pleural cavities normal. Spleen not palpable. Capillary pulse absent; pulse regular; easily compressible.

November 4, 1907: Examination for first time reveals a diastolic murmur heard best over aortic area and transmitted downward; also a capillary pulse in the finger nails.

November 18: Spleen distinctly palpable for first time.

November 25: Patient complains of severe sticking pain in left side and shoulder, made worse on deep breathing.

December 2: Sudden attack of severe pain in left hypochondrium under costal arch, associated with marked tenderness over spleen. Coagulation time of blood 3·5 min.

December 25: Suffering from an acute attack of pneumococcus tonsilitis. Very tender, swollen submaxillary gland; this disappeared without suppuration. Petechial hemorrhages occurred for the first time.

From this time on the patient failed rapidly, the opsonic index going far below normal and the leucocytosis going higher. A progressive anemia of the secondary type was now marked. The hemoglobin went to 38 per cent before death. The pulse with the patient in bed remained relatively low. It was always regular and showed no evidence of myocardial degeneration, until shortly before death when dilatation and arythmia of the heart occurred. Died January 22, 1908.

Anatomic diagnosis.—Ulcerative mitral, aortic, and mural endocarditis; multiple, infarcts in the spleen; petechial hemorrhages in the skin; anemia; fibrinous pleuritis and peritonitis; acute tracheo-bronchial lymphadenitis; hyperplasia of the spleen; edema of the lungs; cloudy swelling of the liver, kidneys, and myocardium; hypertrophy of the heart; obliterative fibrous pericarditis; fibrous pleuritis.

The body is that of a well-developed girl; 170 centimeters long. Over the face, forearms, trunk, and buttocks are numerous small reddish, slightly elevated spots measuring 1 to 3 mm. in diameter. Rigor mortis is present and posterior lividity well marked. The skin and mucous membrance of the mouth pale; the abdomen flat. The peritoneal cavity contains about a liter of slightly turbid fluid. The appendix normal. The right pleural cavity contains a small amount of blood-tinged, slightly turbid fluid and is partially obliterated by fibrous adhesions. The left pleural cavity is practically obliterated by fibrous adhesions and contains a small amount of blood-tinged fluid. The pericardial cavity is obliterated by fibrous adhesions. The thyroid gland and larynx are unchanged. The trachea contains a large amount of bloody frothy fluid. The tracheo-bronchial lymph glands are moderately enlarged, soft, and of a grayish-red color on cut surface. On the left lung pleura is rough and mottled grayish red. The upper lobe crepitates freely throughout; the lower lobe feebly. The cut surface exudes a large amount of bloody, frothy fluid. A piece cut from the lower lobe floats on water. The right lung presents similar alterations.

The heart measures 15×12×4 cm. and is much enlarged. The aortic and pulmonary valves are competent to the water test. The mitral leaflets are shrunken and difficult to make out. The whole margin of the mitral valve is the seat of vegetative growths. At one portion anteriorly the vegetations have grown over the mural endocardium of the left auricle. The tricuspid and pulmonary valves are unchanged. The aortic valve has on each leaflet an abundant mass, largely composed of clotted blood, and measuring 5×10 mm. The vegetations have grown from the line of closure forward and at no point is the free margin implicated. The myocardium is grayish red. The wall of the left ventricle measures 18 mm. in thickness and that of the right ventricle 5 mm.

The spleen measures 15×7×5 cm.; capsule, smooth except over the regions about to be described. There are seen externally eight areas, grayish in color and measuring 5 cm. in their longest diameter; they are all depressed and contain a grayish-white center; surrounded by a zone of red. On section they are wedge-shaped and grayish white in color, not softened, surrounded by hyperemic splenic tissue.

The tonsils are small; the tongue, esophagus, mesenteric glands, intestines, and stomach are unchanged. The liver is grayish red in color and the lobular markings are distinct. The central veins are dilated. The gall-bladder contains thick black bile. The pancreas is normal. The kidneys, the uterus, ovaries, and tubes are normal.

Microscopic examination.—In the lungs are areas in which the alveoli are filled with blood and leucocytes. The colloid is increased and the cellular elements decreased in the thyroid. The lymph glands are hyperemic. The myocardium shows a moderate amount of fatty infiltration. There is hyperemia in the spleen and anemic infarcts with a hemorrhagic zone at the border. In the liver there are marked congestion and slight fatty changes. In the pancreas are seen areas of connective tissue proliferation between the lobules. There is marked hyperemia and connective tissue proliferation in the kidneys. There are no noteworthy changes in the ovary.

Bacteriologic examination.—The bile, serum, blood, peritoneal fluid, right pleural exudate, and vegetations were examined. In cover-glass preparations the bile is sterile; in the serum, blood, peritoneal fluid, right pleural exudate, and emulsion of the vegetations, are seen Gram positive cocci arranged in clusters and chains and in twos, and in the vegetation emulsion is a short Gram negative bacillus. Cultures prove the bile sterile and give a proportionately large number of pneumococci and a few staphylococci (aureus) from serum, blood, peritoneal fluid, right pleural exudate, and vegetation emulsion; also the streptococcus pyogenes from the blood, pleural and peritoneal fluids, and vegetation emulsion; bacillus mucosus from the vegetation emulsion.

Animal experiments.—Two rabbits injected with the pneumococcus 311^{IX} developed endocardial lesions; in one the tendency seemed to be toward healing; the other died with ulceration of the aorta just beyond the cusps associated with multiple infarcts of kidneys and lungs. The blood cultures of the former were sterile, while in the latter they gave a coccus of the same characteristics as the one inoculated. A third animal died five days afterward without lesions and the coccus isolated from the blood had lost its special chaarcteristics, behaving exactly as ordinary pneumococci. Four rabbits recovered without lesions after inoculation with large doses. The reason for the high percentage of recoveries with this strain is probably a low grade of virulence at the time of inoculation (90 days after isolation) in spite of the fact that the special characteristics were still retained. The virulence could not be increased at this time.

Characteristics of the coccus.—The characteristics of the organism isolated from the blood before and after death may be summarized as follows: It grows in colonies in the fibrin clot in the blood cultures, the liquid portion remaining clear for 48 hours. This characteristic I have not observed in pneumococci isolated from the blood in lobar pneumonia. In plain and dextrose broth the endocarditis organism produces a flocculent sediment consisting of large masses of closely adherent bacteria. Some of the masses attach themselves so tightly to the side of the test-tube that vigorous shaking fails to dislodge them. The broth remains perfectly clear. The strain isolated in the third blood culture lost this adhesive property on injection into rabbit after four months of artificial cultivation on blood agar, and cultures from the heart's blood grew like pneumococci from the blood in pneumonia; at the same time the strain assumed a moderate virulence and a corresponding lessened susceptibility to phagocytosis. The strain isolated in the tenth blood culture two months later still possesses this interesting characteristic to a moderate extent, now for over nine months since. Injections into animals of this strain have thus far failed to bring about any change.

In blood-agar plates the organism produces with a few exceptions small but somewhat variably sized colonies with a greenish zone resembling the pneumococcus except in that the production of green is not so marked. At no time was there noted hemolysis either of homologous or heterologous blood. In four of six cultures the blood-agar plates showed two varieties of colonies, a larger variety with a green zone and a smaller with no change in the media. Both consisted of Gram positive diplococci. Subcultures from the larger colonies always produced growths exactly like the original. The smaller colonies usually produced the smaller colonies, but sometimes the larger colonies also. At first it was thought that possibly we were dealing with a mixed infection but the fact that both varieties were Gram positive diplococci giving the same reactions and that the smaller variety sometimes produced the larger colonies led to the conclusion that they were the same organism, the ones producing the smaller colonies being less vigorous. Analogous observations have been made with respect to strains of pneumococci isolated in pneumonia and after cultivation on the same media for a long time. Upon blood-agar slants this particular strain had a marked tendency to adhere to the surface so that it would be necessary to tear the surface of the media to dislodge them. The bacteria composing the colonies were so adherent that an emulsion for opsonic work could be prepared only with great difficulty. In the water of condensation there appeared clumps of bacteria while the fluid remained clear. This property was accentuated in the successive blood cultures and lasted longer in the tenth strain than in that obtained in the third blood culture. When first isolated the organism fermented inulin slowly, but soon lost this power. It was not dissolved by ox bile. A definite capsule was demonstrated when first isolated in three of the blood cultures, but in two blood cultures the capsule was absent.

Case 341.—Woman, 35, mother of three children; no history of acute rheumatism or other recent infection. Complains of shortness of breath and a growing weakness for past year which has become so severe that she had to give up her work; is no longer able to lie flat in bed on account of dyspnea. Frequently has feeling of fulness and sense of oppression over heart and pain when especially short of breath. Swelling of feet toward end of day; subsides during night. Dry, hacking cough, occasionally spits a little blood; sputum lumpy. Has had no chill but thinks she has more or less fever every day. Menstruation more frequent than usual but less. Marked pallor; cyanosis; fingers slightly clubbed; no petechiae; pulsation of pericardium, systolic

thrill; loud presystolic murmur at apex in axillary line; pulmonic second sound accentuated; left auricle dilated; lungs and pleural cavity normal. Liver edge two fingers below costal arch; no edema; hb. 80 per cent, leucocytes 14,000. Blood pressure 130. Temperature 100.5°. In hospital for two months; temp. never went above 100° and to that point only twice. The leucocyte count became normal; pulse practically normal when in bed but high when walking about. Four months later she returned. There was no marked change in the heart; general condition, however, worse in spite of better care. Hemoglobin now 70 per cent; leucocytes 8,900; temp. 99.8 . Four months later gradually worse. Blood culture 30 colonies per c.c. of pneumococcus with the special characteristics common to the other strains isolated from cases of endocarditis, adhering to the surface of blood agar, growing in clumps and short chains, but susceptible to phagocytosis and not virulent for animals. (Patient still living when last heard from.)

Animal experiments.—May 13, 1908: Large Belgian hare. 10 c.c. 24-hour blood-broth culture intravenously; 10 c.c. 24-hour blood-broth culture intraperitoneally and subcutaneously.

May 14: Seems ill. Blood cultures.

May 15: Average of 8,000 green colonies per c.c. of blood. Seems better.

May 16: Seems quite ill.

May 18: Found dead. Slight fibrous deposit at site of subcutaneous injection. No peritonitis. Fibrinous pericarditis; vegetative endocarditis of aortic and mitral valves; mural endocarditis of left ventricle and right auricle; the vegetation of the latter has grown down and become adherent to the mitral valve. Smears from the pericardium show large numbers of Gram positive diplococci many of which are within leucocytes in various stages of disintegration. 250 colonies per c.c. of blood on blood-agar plates. Cocci obtained by centrifugation resisted phagocytosis by normal human and homologous rabbit's leucocytes in homologous serum Cultures from the pericardium, the vegetations, and the heart blood gave pure cultures of the coccus inoculated. After cultivation for two days the cocci were taken up quite freely by human leucocytes in human serum and rabbit's leucocytes in rabbit serum. Subsequent injections of large doses of the coccus in rabbit and guinea-pig produced death from pneumococcemia, the coccus now having the characteristics of ordinary pneumococci.

May 11, 1908: Medium sized albino. 5 c.c. 24-hour milk culture intraperitoneally.

May 12: Seems perfectly well. Peritoneal fluid watery, free from bacteria; few leucocytes and endothelial cells.

May 15: Perfectly well.

May 16: Ill. Blood culture.

May 18: Well. Blood culture sterile.

May 19: Perfectly well.

May 20: Seems well; chloroformed. Several small thrombi in portal vein.

May 22: In cultures of blood growth of Gram positive diplococci which resemble the coccus inoculated; they adhere to the surface of the blood agar, are susceptible to phagocytosis in human serum by human leucocytes. The growth is less vigorous and the production of green about the colonies on blood-agar plates is less marked than originally.

Case 353.—Banker, 48, married; Dr. Clarke's patient. Inflammatory rheumatism 23 years ago. During 10 subsequent years one or two attacks annually. Joints

much inflamed during the attacks. Tonsilitis as a child. Six years ago an attack of tonsilitis and grippe. Pyorrhea for past four years. Bronchitis relatively frequent. One year ago pain over epigastrium, icterus, and clay-colored stools (once only). During last year much mental but little physical depression. Nearly a year ago dyspnea noticed on going up stairs, with pain over heart and, soon, attacks of angina pectoris. Fever and chills began nine months ago, temperature during the evening 99½ to 103°; usually subnormal in morning. Repeated chills and chilly sensations for past four months. Weight reduced from 200 to 130 pounds. Universal petechiae noticed one week ago. Went to bed nine months ago and has been there most of the time since.

Physical examination.—Much emaciation, sallow and listless. Multiple petechiae—disappearing now—over entire body. Slight mitral murmur, systolic; heart dulness increased slightly to left. Gums spongy and teeth in poor condition, one upper incisor loose. Blood culture gives about 16 colonies to 1 c.c. blood on blood agar and potato. The injection of 50 million of the homologous coccus had no apparent effect upon the patient's condition. Death about 11 months after the definite symptoms appeared.

The coccus isolated from the blood produced typical green colonies upon blood-agar plates. It resembled in every way the colonies of pneumococci from the blood in pneumonia, except that on blood-agar plates as well as slants, the cocci grow tightly to the surface of the medium. This property was lost in one week. It also had the property of growing in clumps in the fibrin clot of the broth cultures and only slowly produced turbidity of the fluid portion of the broth. The early subcultures fermented inulin slowly when grown on litmus inulin agar; this power was promptly lost. The organisms were not dissolved by ox bile; capsules demonstrable by the Welch and Buerger method, and stained well by Gram's method. In milk and broth the coccus tended to grow in clumps of from 50 to 200, but there were also single and short chains of diplococci. The second subculture in broth was freely susceptible to phagocytosis in normal blood. This was also true of the coccus when grown on agar slants and suspended in salt solution.

Animal experiments.—Four rabbits inoculated with large doses of the first daughter culture in blood broth, 24 hours old. Rabbit 168 received 3 c.c. intravenously and 3 c.c. intraperitoneally, dying five days later, and typical encapsulated diplococci were isolated from the blood in fair numbers. Rabbit 169 was given 3.5 c.c. intraperitoneally and 3.5 c.c. subcutaneously; seemed perfectly well four days later, but died in 10 days from a pneumococcemia. A small subcutaneous abscess was found at the site of injection but no peritonitis, pericarditis, or endocarditis.

Rabbit 167, June 19, 1908: Injected 3 c.c. intravenously and 3 c.c. intraperitoneally and 3 c.c. subcutaneously.

June 20: Seems fairly well.

June 23: Seems to have recovered.

June 31: Seems fairly well but is losing weight.

July 19: Large abscess at point of subcutaneous injection but seems fairly well except labored breathing upon exertion or when handled. A sharp systolic murmur can be heard with the stethoscope over the heart.

July 20: Chloroformed. Massive adhesive and fibrinous pericarditis; vegetative endocarditis of mitral valve; subcutaneous abscess. No peritonitis. Smears from the crushed portion of the vegetation and from material from the pericardium show a

arge number of Gram positive diplococci while those from the heart's blood and peritoneal fluid are sterile. Cultures from the heart's blood, vegetations, peritoneal fluid, and pericardium on blood agar and in broth are sterile.

Rabbit 170, June 19, 1908: 3 c.c. intravenously.

June 20: Blood cultures show moderate number of pneumococci. Seems fairly well.

June 23: .Is losing weight but otherwise seems fairly well.

June 29: Found dead.

Autopsy shows a huge fibrinous pericarditis and multiple abscesses of the liver which are situated about radicles of the portal vein and from which thrombi spring and extend backward. The endocardium is smooth. Smears from the pericardium and the thrombi show a large number of Gram positive diplococci some of which are within leucocytes. No evidence of phagocytosis in the blood. Cultures on blood-agar plates show typical green colonies.

Case 359.—Girl, 18 years old, under the care of Dr. Billings. Was always delicate; usual diseases of childhood; menses at 14; regular as a rule; frequent nose bleed since 12; worked hard at her studies last year, and became weak both physically and nervously. At present complains of weakness, anorexia, restless sleep, dyspnea after exertion, black spots before eyes on rising suddenly. Had tonsilitis six or seven months ago, was in hospital for a week, but felt well afterward. No history of rheumatism. Hemoglobin 50 per cent, reds 3,560,000; whites, 21,000. Urine normal. Pulse small, 140 per minute; respirations 30; temperature 101.6°. Diffuse apex beat; left border of heart 11 cm. from center of sternum; left base to the second rib and right border of cardiac dulness to the center of the sternum; very loud systolic rough murmur at the mitral and apex areas, transmitted to the axilla and back; over center of sternum a churning murmur with both actions of the heart; pulmonic second sound greatly accentuated and louder than the aortic which is also somewhat accentuated. Spleen palpable at costal arch. Liver not enlarged. Abdomen scaphoid. No purpuric spots; no glandular enlargements. Blood cultures gave 500 colonies of pneumococci per c.c. of blood, resembling those isolated from other cases of endocarditis. The cocci grew in the fibrin clot of the blood, were susceptible to phagocytosis, and had practically no virulence to animals. The colonies at first were tightly adherent to the surface of blood-agar slants and produced a viscid sediment in broth and no uniform turbidity. Both these properties were soon lost.

The patient was placed at absolute rest in bed but gradually grew worse and died two months later. Three times she was injected with approximately 75 million of dead pneumococci (own); an improvement followed each injection lasting 24 hours.

Animal experiments.—The injection of huge doses intravenously and intraperitoneally in three rabbits and one guinea-pig after the special characteristics were lost failed to produce endocarditis. One rabbit died of a pneumococcemia without peritonitis. The rest recovered promptly.

CHARACTERISTICS OF THE COCCI STUDIED.

The more important characteristics only are summarized here:

The cocci always produced more or less greenish discoloration around the colony upon blood-agar plates but never a zone of hemolysis. The variation in this respect was great; usually, however,

the strains from endocarditis produced less green than the strains from pneumonia.

Most of the strains soon after isolation fermented inulin to a mild degree, a property which all soon lost. Pneumococci from the sputum in pneumonia ferment inulin more rapidly than those isolated from the blood. They also retain this property longer than the latter when cultivated upon artificial media. Hence it would seem that the lessened fermentative powers of the endocarditis cocci might be looked upon as owing to a long residence in the blood.

When first isolated in broth they grew in the fibrin clot in large colonies, a form of growth I did not observe in the study of over 300 strains of pneumococci from cases of pneumonia. The endocarditis cocci grew in clumps in the broth and produced a diffuse turbidity slowly, and upon agar slants the colonies grew more or less tightly to the surface. The stained specimens gave diplococcus forms and chains. The chain formation as well as the property of growing in clumps in broth, and of adhering tightly to the surface of blood-agar slants, disappeared after cultivation for a variable period, depending upon the degree to which these special characteristics were present in the beginning. The more chronic the course of the infection and the later in the course of the disease the organism was isolated, the more marked and the longer these special characteristics lasted.

By cultivation on artificial media all the strains change gradually into typical lanceolate diplococci, often capsulated, growing as typical pneumococci in broth and on blood-agar slants. On animal inoculation this modification often occurred abruptly. It has been impossible to so modify strains of *Strept. viridans* which these organisms much resemble.

Agglutination.—The cocci used in the agglutination tests (see Table 5) were grown upon the surface of plain agar slants and suspended in salt solution. The results given were read 12 hours after the mixtures were made. The table shows a high degree of agglutinating power of the serum from the cases of endocarditis with respect to pneumococci isolated from endocarditis, a moderate power for two strains of pneumococci isolated from the sputum and blood of two cases of pneumonia, and no agglutinating power in the dilutions used, for *Strept. pyogenes*, *Strept. mucosus*, and *Strept. viridans*. Pneumonic

serum, obtained the day after crisis, in the dilutions used, had approximately the same agglutinating power over pneumococci isolated from pneumonia and endocarditis but none over the streptococci. Normal serum showed a slight agglutinating power for two strains of pneumococci isolated from endocarditis while for pneumococci from pneumonia and the streptococci it had no agglutinating power. Similar results were obtained with other sera and strains obtained from cases of endocarditis.

TABLE 5.

COMPARATIVE AGGLUTINATING POWER OF NORMAL, ENDOCARDITIC, AND PNEUMONIC SERA ON STREPTOCOCCI AND PNEUMOCOCCI.

ORGANISM	NORMAL SERUM		ENDOCARDITIC SERUM		PNEUMONIC SERUM		NORMAL SALT SOLUTION
	1–100	1–500	1–100	1–500	1–100	1–500	
Strept. pyogenes	o	o	o	o	o	o	o
Strept. mucosus	o	o	o	o	o	o	o
Strept. viridans	o	o	o	o	o	o	o
Pneumococcus from sputum in pneumonia	o	o	++	o	+	o	o
Pneumococcus from blood in pneumonia	o	o	++	o	+	o	o
Pneumococcus 311, from blood of patient with endocarditis	+	o	+++	++	+	o	o
Pneumococcus 292, from blood of patient with endocarditis	o	o	++	++	+	o	o
Pneumococcus 293, from blood of patient with endocarditis	+	o	+++	++	+	o	o

THE ENDOCARDITIS COCCI, MODIFIED PNEUMOCOCCI.

That we are not dealing with *Strept. pyogenes* in these cases, even though the early smears show chains, is certain for obvious reasons. That the strains should be looked on as belonging to the pneumococcus group and as having undergone environmental modification and not be regarded as strains of *Strept. viridans* seems warranted because all the strains have been changed into typical pneumococci by prolonged artificial cultivation or animal inoculation; because they fermented inulin when first isolated; because the serum from the cases had a high agglutinating effect for known typical pneumococci and not for either *Strept. viridans* or other streptococci; because the environmental modification grew more marked as the infection progressed, and because the opsonic power of the serum of the cases showed a decided specificness for the various endocarditis strains as well as for pneumococci from pneumonia, but not for streptococci.

RESULTS OF OBSERVATIONS ON OPSONIC INDEX, THERAPEUTIC INOCULATION, LEUCOCYTES, AND SERUM, IN THE CASES OF PNEUMOCOCCUS ENDOCARDITIS.

The observations recorded in Chart 2 were made upon case 311 (see page 259), a case of pneumococcus endocarditis of the mitral valve which began insidiously on top of an old endocarditis the result of rheumatism, and ran a course of six months or longer. The case offered an excellent opportunity for a study of the blood in various ways because the infection continued for a long time without marked variations. During three months this study was interrupted only three times by acute phenomena, twice on account of infarctions (as shown at the post mortem), and once by an attack of pneumococcus tonsilitis.

Repeated blood cultures were made to learn whether bacteremia in endocarditis is constant and to determine the number of cocci in the blood; and also to learn whether the inoculation of dead bacteria would have any influence upon the number of cocci in the blood. Ten cultures were made; of these all but the first showed pure cultures of pneumococcus. Cultures in broth sometimes proved sterile, but upon agar plates from 35 to 50 colonies per c.c. of blood developed. There was a steady diminution in the number of cocci during the period of the first eight injections, but the patient showed no signs of improvement; instead he slowly grew worse. This diminution was probably only apparent owing to greater clumping of the bacteria as there was noted an increased agglutinating power of the serum in this case as time went on. There seemed to be no causal relation between the number of leucocytes, the opsonic index, and the number of bacteria in the blood. In the beginning the opsonic index was tested with respect to three strains of pneumococci, one from pneumonia, one from a similar case of endocarditis and the homologous strain. The results corresponded closely. The results given in Chart 2 were obtained with the strain from another case of endocarditis which was used because it was especially adaptable for the work. The Wright method as well as dilution method gave similar results.

The facts brought out by the study of the opsonic index, the leucocytes, and the temperature curve in this case may be summarized briefly as follows: The average opsonic index before the injections were

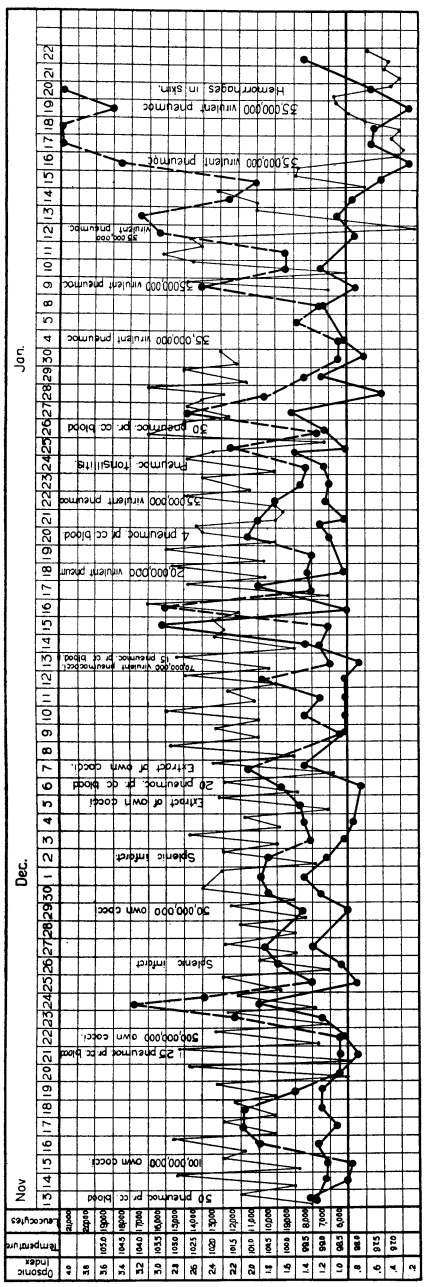

CHART 2.—Opsonic Index, Temperature, and Leucocyte Curves in Case 311. Solid heavy line = pneumococco-opsonic index; solid fine line = temperature; broken heavy line = leucocytes.

begun was above normal, hence a lack of opsonin could hardly be held responsible for the continuance of the infection. The injection of the homologous as well as of dead virulent pneumococci as indicated on the chart caused a rise in the opsonic index in nearly every instance. The rise was usually marked upon the day following, going still higher on the second day, and then usually in one or two days more the index dropped to a point lower than previous to the injection. A rebound sometimes occurred. A negative phase was not observed; if there was any it occurred inside of 24 hours. Dead virulent pneumococci were injected because it was thought that the homologous organism might have lost the power to stimulate properly the immunizing mechanism of the host. They produced a greater local and general reaction. The injection of 70 million on December 13 was more effectual in raising the opsonic index and leucocytosis than the injection of 500 million killed cocci of the patient's strain on November 2. The injection of extracts in salt solution of the homologous organisms upon two occasions was followed by a drop instead of a rise in the opsonic index.

Other things being equal, the rise in the leucocytes and in the opsonic index was roughly proportionate to the amount injected. Early the rise and fall in the opsonic index and leucocytes ran parallel, later, as the general condition of the patient became worse, the opsonic index falling, the reverse seems to have been true.

Previous to the time when the temperature became subnormal and the patient moribund there was a distinct or slight rise in temperature as the opsonic index and leucocytes rose after seven inoculations and a drop after three.

There was no marked change in the patient's general condition one way or the other until later, the opsonic index then being far below normal, when for 24 hours following the injection there was a rise in the opsonic index, and the patient seemed to be more comfortable, the pulse stronger, and the general condition better. That there may be a close relation between the temperature and the opsonic index is indicated by the observations on December 24 and 25. The index on these days was taken when the temperature was the highest at midnight, and lowest, four hours later. The results show a decided drop in the index with the drop in the temperature which was associated

with profuse perspiration. Hence great variations in the index may occur in short periods of time. We note further the agonal rise in the index, the tendency of the index to drop to a lower level after the temporary rise than it was previous to the injection, and the drop after the injection of the extracts.

From a consideration of all the facts it would seem that the injection of bacteria had no curative effect in this case. Opsonification and phagocytosis seem to have been of no importance in combating the infection in spite of the fact that we were dealing with a coccus which was susceptible to phagocytosis on cultivation and of low virulence to animals. Now a study of Table 6 shows that normal blood (washed

TABLE 6.

THE COMPARATIVE PNEUMOCOCCIDAL VALUE OF NORMAL AND ENDOCARDITIS BLOOD.

MIXTURES *	PHAGOCYTOSIS 15 Min.	NO. OF COLONIES IN BLOOD-AGAR PLATES		
		At Once	24 hr.	48 hr.
1. Washed normal blood + normal serum + Pn. 311	3.	780	5	0
2. Washed normal blood + 311 serum + Pn. 311	3.4	550	25	0
3. Washed 311 blood + normal serum + Pn. 311	2.8	350	134	10
4. Washed 311 blood + 311 serum + Pn. 311.	4.6	800	4,000	∞

* Two sets of tubes were made, one for the study of phagocytosis, the other for the study of pneumococcidal effect, each containing equal parts of serum and washed leucocytes and broth culture of the coccus. The washed normal blood contained 20,000 leucocytes per c.mm. and 311 blood 22,000. The colonies represent the bacteria per loop plated out at the time indicated. The experiment was performed on a day when the leucocyte count was 15,000.

∞ = uncountable.

normal leucocytes + serum) and the patient's washed leucocytes in normal serum had a marked destructive power over the coccus from the patient's blood, while the leucocytes in the patient's serum had no such power. This must be ascribed to loss of the power of the leucocyte in the presence of this particular serum of digesting the bacteria and not to the lack of phagocytosis. Other tests showed that the organism grew well in the patient's serum. The lack of destructive power of the patient's blood is not specific for the homologous coccus because other strains grew invariably in the patient's defibrinated blood, while in normal defibrinated blood, with the same number of leucocytes, there was destruction.

In a previous paper[1] I have shown that the leucocytes in croupous

[1] *Jour. Infect. Dis.*, 1906, 3, p..683.

pneumonia and other acute infections with an active leucocytosis may have a higher phagocytic value for pneumococci than normal leucocytes. A study of the phagocytic value of the leucocytes in this case is shown in Table 7 which reveals that the opsonic index usually cor-

TABLE 7.

THE COMPARATIVE PHAGOCYTIC POWER OF NORMAL AND ENDOCARDITIS (311) LEUCOCYTES.

MIXTURES*	PHAGOCYTOSIS IN 15 MINUTES							
	Nov. 22	Nov. 26	Nov. 30	Dec. 12	Dec. 25	Dec. 26	Dec. 27	Dec. 30
Washed normal blood + normal serum + Pn. 292	3.4	2.7	1.9	2.	2.4	4.5	3.	5.
Washed normal blood + 311 serum + Pn. 292	3.1	3.	2.4	2.4	3.9	5.9	3.4	4.
Washed 311 blood + normal serum + Pn. 292	3.5	4.3	2.	3.	4.7	3.5	2.8	5.5
Washed 311 blood + 311 serum + Pn. 292	3.	3.9	2.8	3.6	6.	3.	4.6	3.4
Leucocyte Count on Dates Indicated	6,500	10,000	8,500	11,000	12,500	7,500	15,000	6,800

* The number of leucocytes was controlled by counts of the washed blood. Equal numbers of normal and patient's leucocytes were used. Differential leucocyte counts showed the proportion of polynuclears to be approximately the same in both. The strain used in these tests was obtained from another but similar case of endocarditis. It had been cultivated for a longer time and gave an even suspension. Controls with the patient's own organisms gave the same results.

responds when normal and patient's leucocytes are used. Two exceptions occur: November 26 and December 26. On these dates the index is high with normal leucocytes and low with patient's leucocytes; when the index is low with the former it is always low with the latter. The most significant point in this connection is that the patient's leucocytes are less active or equally as active as normal leucocytes on the date when there is no leucocytosis and more active when there is leucocytosis. This is true usually both with normal and patient's serum. In this case there is, then, not only a difference in the phagocytic activity of leucocytes upon different dates but, what is more significant, a lack in the power of destroying the engulfed bacteria. The increased phagocytic activity during leucocytosis is not associated with an increased destructive power.

Chart 3 gives a summary of the observations on Case 293. The chart shows that the injection of the homologous dead organisms was followed by a rise in the leucocytes; the injection of a smaller dose caused no marked changes on one occasion, a pronounced increase in the opsonic index in another, while a marked decrease followed the third injection which contained a large dose. The temperature for

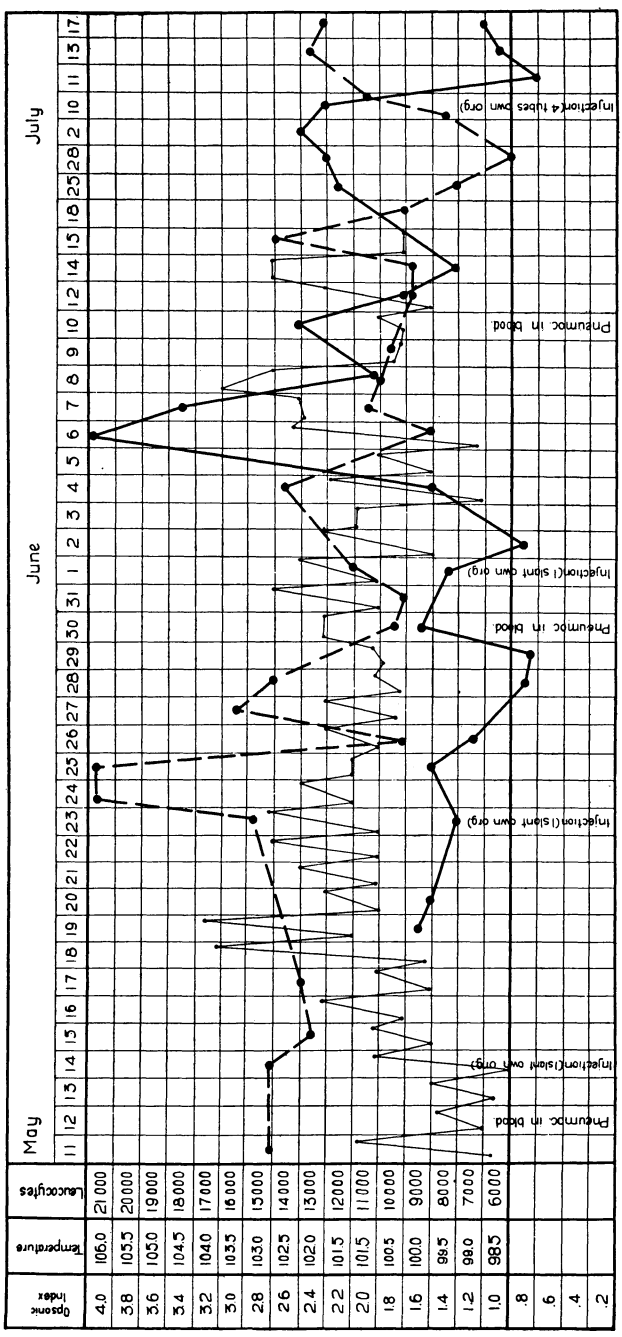

CHART 3.—Opsonic Index, Temperature, and Leucocyte Curves in Case 293. Solid heavy line = pneumococco-opsonic index; solid fine line = temperature; broken heavy line = leucocytes.

several days after the injections showed a moderate drop after two, a definite rise after one, and no apparent change after the other injection. There was, therefore, no constant relation between the opsonic index, the leucocytes, and the temperature following the injections in this case. The exceptionally high opsonic index of 4.0 upon June 6 may have been owing to autoinoculation from the submaxillary aneurysm rather than the result of the injection of bacteria.

Table 8 shows that the patient's leucocytes were more active than normal leucocytes toward the strain isolated in this case as well as

TABLE 8.

INCREASED PHAGOCYTIC POWER OF PATIENT'S (293) LEUCOCYTES AS COMPARED WITH NORMAL LEUCOCYTES.

MIXTURES	PHAGOCYTOSIS IN 15 MINUTES	
	Normal (7,000 Leucocytes per c.mm.)	293 (7,500 Leucocytes per c.mm.)
Normal serum + Pn. 293	6.	9.
Normal serum + Pn. 245	5.5	11.4
Serum 293 + Pn. 293	5.1	8.7
Serum 293 + Pn. 245	4.7	9.

toward pneumococcus from the sputum of a case of pneumonia. The patient's leucocyte count on the day of the experiment was 12,000. The homologous organism grew readily in the patient's serum which was agglutinating in dilutions of 1 to 1,000, but grown in the patient's serum for 24 hours the strain showed a marked diminution in susceptibility to phagocytosis, especially in the patient's blood.

Chart 4 is a summary of the more important observations on another case (292) of pneumococcal endocarditis of the pulmonary valve in which no source of infection could be found and in which no previous heart lesion was present. The duration probably was over a year and death seemed to be caused by a terminal streptococcemia. The injection of 2,500,000 dead homologous cocci was followed by a rise in the opsonic index from 0.55 to 1.1 in two days. The great drop in temperature and pulse shown in the chart at this time may have been due to a large dose of morphine given, because the second injection which also raised the opsonic index was not associated with such drop. There was decided symptomatic improvement in the patient's condition for two days following the first injection. There was no such marked change after the second injection, probably because of the

invasion of streptococci which may have been the immediate cause of death. On the other hand the leucocytes, temperature, and pulse rapidly went up while the opsonic index went down. The strepto-cocco-opsonic index at the time of death was also very low, whereas it had been nearly normal.

Table 9 gives the results of a study of the serum and leucocytes of case 353. Pneumococcus 353 is the coccus isolated from this case,

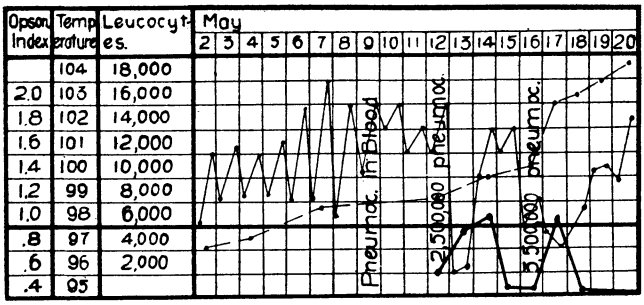

CHART 4.—Opsonic Index, Temperature, and Leucocyte Curves in Case 292. Solid heavy line = pneumococco-opsonic index; solid fine line = temperature; broken fine line = leucocytes.

TABLE 9.

THE PHAGOCYTIC VALUE OF THE LEUCOCYTES AND OPSONIC POWER OF THE SERUM FROM A CASE OF ENDOCARDITIS (353).

Mixtures	Agglutination	Phagocytosis in 15 Min.
Normal blood + normal serum + Pn. 353	No Agglutination	5.
" " + patient's " + Pn. 353	Marked "	6.2
Patient's " + normal pooled serum + Pn. 353	No "	2.4
Patient's " + patient's serum + Pn. 353	Marked "	2.
Normal blood + normal pooled serum + Pn. 291	No "	4.85
" " + patient's serum + Pn. 291	" "	4.5
Patient's " + normal pooled serum + Pn. 291	" "	4.3
" " + patient's serum + Pn. 291	" "	5.

while pneumococcus 291 is an ordinary pneumococcus isolated weeks previously from the blood of a case of lobar pneumonia. The blood was obtained five days before death and two days after the injection of 50 million heated homologous cocci. The opsonic index for the homologous cocci is 1.2 when obtained with washed normal blood, and 0.9 for strain 291. On the other hand when the patient's leucocytes are used the total amount of phagocytosis is less than half as great and the opsonic index is .8 for the homologous coccus and 1.1 for strain 291. The patient's leucocytes are as active phagocytically as normal leucocytes for strain 291 (see Table 5). The difference is not

the result of agglutination because there is the same amount of agglutination when normal and patient's leucocytes are used in the presence of patient's serum and there is no agglutination in the presence of normal serum. Further, the patient's serum was strongly agglutinative of pneumococcus isolated from endocarditis for which the leucocytes were as active phagocytically as normal leucocytes, the opsonic power of the patient's serum in both instances being high. It would seem therefore that the diminished phagocytic activity of the patient's leucocytes is closely related to the homologous organism and that the patient's serum is less opsonic than normal serum. There is then in this case a specific diminution of the phagocytic power of the patient's blood with respect to the organism causing the infection. Table 10

TABLE 10.

COMPARISON OF GROWTH OF PNEUMOCOCCUS 353 IN SERUM 353 AND NORMAL SERUM.

MIXTURES	AGGLUTINATION	NUMBER OF COLONIES IN BLOOD-AGAR PLATES	
		At Once	20 Hours
Normal serum + Pn. 353	No agglutination	4,300	3,000
Patient's " + " "	Marked "	4,200	7,500
Normal " + " 311	No "	5,800	3,100
Patient's " + " "	" "	6,500	8,900

shows that the pneumococcus isolated from the patient, as well as a strain from another case of endocarditis, grows more readily in the patient's serum than in normal serum, there being more than twice the number of colonies at the end of 24 hours in the patient's serum in spite of the marked agglutination.

SUMMARY OF ANIMAL EXPERIMENTS WITH PNEUMOCOCCI.

Table 11 gives a summary of the animal experiments with pneumococci. It shows that endocarditis developed in five of ten animals inoculated before the special characteristics of the pneumococci were lost. In none of these were the valves injured before inoculation. Pericarditis developed in six cases; in two, endocardial lesions were absent while pericarditis developed and also beginning thrombosis of the portal vein. The apparent affinity of these organisms for the endocardium and pericardium is quite apparent. Pericarditis developed quite independently of the place of inoculation. The right heart was involved only once; in the other cases the mitral was affected

TABLE 11.

SUMMARY OF ANIMAL EXPERIMENTS WITH PNEUMOCOCCI FROM ENDOCARDITIS.

Animal	Organism Inoculated	Days Cultivated Since Isolated	Special Characteristics	How Inoculated	Postmortem Findings	Bacteriologic Findings	Remarks*
Rabbit	311ix	90	+	5 c.c. milk culture intravenously	Healing aortic, pulmonary, and mural endocarditis; localized pericarditis	Smears from endocardium and pericardium show Gram positive diplococci	Chloroformed 21 days after injection. Seemed quite well
"	311ix	90	+	15 c.c. intravenously 24-hr. blood-broth culture	Ulcerative aortitis; infarcts of kidney and lungs; pleuro-pericarditis	Organism recovered	Death in 28 days from inoculation
"	311ix	90	+	5 c.c. blood-broth culture	No localization, no peritonitis	Smears and cultures yield typical lanceolate diploc. which have lost their special characteristics	Death in 5 days from pneumococcemia
"	341ix	3	+	10 c.c.† intravenously 10 c.c. intravenously and subcutaneously	Vegetative endocarditis of aortic and mitral valves; endocarditis left auricle and left ventricle. Fibrinous pericarditis; no peritonitis	Coccus of same characteristics isolated from blood pericardium, vegetations	Death in 11 days after inoculation
Guinea-Pig	341	2	±	3 c.c. into heart 3 c.c. intraperitoneally	Death without lesions in 48 hr.	Pneumococci isolated like those from blood of pneumonia	Organism when inoculated had lost part of former characteristics
Rabbit	341	3	+	5 c.c. intravenously 4 c.c. into heart 3 c.c. into peritoneum	Death in 48 hours from peritonitis and pneumococcemia	Pneumococci typical of those from blood of pneumonia	
"	353	2	+	3 c.c. intravenously 3 c.c. intraperitoneally 3 c.c. subcutaneously	Massive, adhesive. and fibrinous pericarditis; vegetative endocarditis of mitral valve; subcutaneous abscesses;. no peritonitis	Smears from vegetations and pericardium show abundance of Gram positive diplococci	×
"	353	2	+	3 c.c. intravenously Injection of 5 c.c.organism 311ix 4 weeks previous	Pericarditis; portal thrombosis; no endocarditis	Pure cultures of pneumococci from heart's blood. epicardium and portal vein	Death in 48 hours
"	353	2	+	3 c.c. intravenously 3 c.c. intraperitoneally	No anatomic lesions	Typical encapsulated diplococci	
"	353	2	+	3.5 c.c. intraperitoneally 3.5 c.c. subcutaneously	Died in 48 hours	Many encapsulated pneumococci in blood	
"	359	7	o	10 c.c. intravenously 7 c.c.intraperitoneally	Chloroformed 2½ months later. No lesions	Blood cultures sterile	
"	359	7	o	10 c.c. intravenously 5 c.c. intraperitoneally	do	do	Loss in weight for a time, then recovery
"	359	7	o	7 c.c. intravenously 7 c.c. intraperitoneally	do	do	
Guinea-Pig	359	7	o	4 c.c. intraperitoneally 4 c.c. into heart with injury to valves	Fibrinous pericarditis; beginning endocarditis; thrombosis of portal vein; osteomyelitis	Cultures from heart's blood, pericardium, and thrombus	Animal died 18 days after injection
Rabbit	344	2	+	10 c.c. intravenously 10 c.c. intraperitoneally; 10 c.c. subcutaneously	Serofibrinous peritonitis	Large number of pneumococci in the blood; capsules present	Death 5 days after inoculation
"	292	10	o	10 u. intraperitoneally			

* Only the animals showing the more important lesions are here tabulated. A number which showed a healing endocarditis are not included.

† The number of c.c. of the culture means a 24-hour culture in broth unless otherwise stated.

three times and the aortic twice. The failure to produce endocarditis after the special characteristics of the pneumococci were lost is interesting; of 16 rabbits injected with various strains of pneumococci after return to the usual type none developed endocarditis. In five the injection was combined with injury to the valves; four died of pneumococcemia after the injection of large doses. Three animals were given a second injection and it was found that the first dose had produced a hypersensitiveness just as in animals injected with staphylococcus 334 (see page 251). Only one animal developed lesions by the injection of a relatively small dose. The injection of huge doses was necessary to produce pneumococcemia when the organisms injected have grown in media free from blood or serum; the presence of the latter in broth in large amounts seemed to enhance virulence. Cultures from the animals that died with endocarditis always showed the above-mentioned special characteristics, while those that were obtained from animals dying of pneumococcemia usually showed the organisms to have taken on the normal characteristics. A study of the blood and peritoneal exudate in some of the animals showed that the organisms disappeared rapidly which is in keeping with their susceptibility to phagocytosis. Blood cultures in four of the animals which developed active endocarditis were sterile 1, 2, 3, 4, and 5 days respectively before death or autopsy. The rapid destruction of the organisms even in the cases which later developed endocarditis was associated with apparent complete recovery for a time. The number of organisms in the blood after death was very large. When death occurred as the result of endocarditis or pericarditis it seemed to be due largely to mechanical obstruction to the flow of blood owing to large vegetations and to mechanical interference of the heart's action by the massive pericarditis.

Joint involvement occurred only once in all the animals inoculated.

Three of the strains that produced endocarditis were used to determine whether repeated inoculation would increase their virulence or their power of producing endocarditis. The virulence promptly became greater, death being caused by pneumococcemia, the cocci taking on the usual or normal characteristics. Restored virulence of the cocci was associated with restored resistance to phagocytosis just as I found to be the case with pneumococci isolated from pneumonia.[1]

[1] *Jour. Infect. Dis.*, 1906, 3, p. 683.

GENERAL SUMMARY.

A comparison of the number of times the sides of the heart were involved in the human cases and in the animals is interesting.

	Human	Animal
Left Side (alone)		
Mitral	7 times	4 times
Mitral and aortic	2 "	2 "
Aortic	1 "	1 "
	10 "	7 "
Right Side (alone)		
Tricuspid	2 "	4 "
Pulmonary	1 "	1 "
	3 "	5 "
Right and Left Side		
Aortic and tricuspid	1 "	2 "

It is to be remembered that the animals developed endocarditis without previous lesion of the valves. We see that the left side of the heart is more often involved in both series and proportionately more often in the human than in the animal cases. The mitral and tricuspid valves are more often involved than the aortic and pulmonary valves in both series; the right side proportionately oftener in the animals.

The greater frequency of involvement of the mitral and tricuspid valves, which contain capillaries, may be owing to embolic origin. This origin certainly occurred in one instance in the animals. That the right side should be involved proportionately more often than the left in the animals is in accord with expectations.

In nearly all cases of spontaneous endocarditis the bacteria which gain entrance into the circulation first must pass over the valves on the right side, consequently one would expect this side to be involved more frequently. Virchow suggested that the greater strain on the right heart in the fetus and on the left heart after birth is the cause of the predominance of endocarditis on these sides respectively during the two periods indicated. Now it has been shown that the property of adhering to the surface of agar and of forming clumps in broth of both the pneumococci and the staphylococcus was dependent to a large extent upon the quantity of oxygen present. Hence it would seem that the presence of arterial or oxygenated blood upon the two sides at the different periods may also serve to account for the peculiarities of localization, perhaps not by favoring the growth of the bacteria in question as Rosenbach believed, but by favoring the development of

the special characteristics which make the bacteria better able to attach themselves to the valve because of increased viscidity as well as to lodge in the minute capillaries of the valves because of the tendency to grow in clumps in the presence of abundant oxygen. This explanation is the more plausible because of the importance of these special characteristics in the production of endocarditis in animals.

The results of the observations recorded indicate that the bacteria in question, which seem to be of relatively little virulence in the ordinary sense, are able to maintain their growth in the blood and upon the endocardium, and ultimately cause death, by a process of immunization against the antibodies of the host. That the bacteria were not highly virulent for human beings in the ordinary sense seems certain because the course in all cases was a chronic one, which we would not expect were the organisms highly virulent. Furthermore, the bacteria upon isolation were all susceptible to phagocytosis in human serum by human leucocytes and were without pronounced pathogenic power for animals. Repeated injection made the animal more susceptible to subsequent injections instead of more resistant. The fact that huge doses were necessary to produce endocarditis in animals would seem to have its explanation in the fact that many of the bacteria were needed to make the tissues susceptible or give the bacteria a chance to adapt themselves to the new conditions.

The bacteria grew better and acquired a greater resistance to phagocytosis in the patient's serum than in the normal human serum and normal leucocytic blood had a much greater bactericidal power for the bacteria than the homologous patient's blood of the same leucocyte content. The bacteria consequently seemed to protect themselves by a process of adaptation to the opsonins and other antibodies of the individual host. Thus the opsonic index with normal leucocytes might be high while at the same time it might be low with the leucocytes of the patient. Again normal leucocytic blood might show no greater phagocytosis under comparable conditions than the patient's blood and the former might cause marked destruction of the bacteria and the latter none. Furthermore, normal leucocytes in the patient's serum and patient's leucocytes in normal serum might be actively bactericidal yet the patient's leucocytes and serum would cause no destruction. This acquired resistance to phagocytosis may be closely

dependent on some thermosensitive property of the serum because the staphylococcus and one strain of pneumococcus when grown in the heated serum of the patient remained susceptible to phagocytosis.

The animal experiments showed that the pneumococci if injected in large doses might cause death by pneumococcemia, especially if the animal had been given injections previously. At other times endocarditis might result. This was usually progressive but healing might also result. It must be emphasized that without the so-called special characteristics I was unable to produce endocarditis without previous injury to the valves. This holds good also for the staphylococcus. Then, again, injections in animals may produce no apparent effects.

There are, then, certain special conditions when endocarditis is readily produced without trauma of the valves.

CONCLUSIONS.

That certain bacteria more frequently attack the valves upon the left side of the heart than the right after birth, and the right more often than the left in the fetus, may be owing in part at least to the fact that abundant supply of oxygen favors the development of certain special characteristics of the bacteria that favor the production of endocarditis. Embolic origin may explain the greater frequency of endocarditis of the mitral and tricuspid valves as compared with aortic and pulmonary endocarditis.

A close relation exists between the biological characters of the bacteria and their ability to produce endocarditis in the class of cases observed.

The bacteria isolated, while having little or no pathogenic power to animals and being susceptible to phagocytosis, present definite evidence of being immunized against the antibodies of the individual host, thereby perhaps overcoming the resistance of the latter.

The injection of dead bacteria in endocarditis has little or no influence upon the course of the disease. Late in the course temporary improvement may follow the injections.

Blood cultures are the best means of making an early diagnosis in acute endocarditis. They should always be made for the identification and study of the infecting organism as well as for prognostic reasons.